AKI risk biomarkers may be ‘as early as it gets’

Karen Lusky

June 2015—Last fall, the FDA cleared Astute Medical’s NephroCheck to pinpoint critically ill adults likely to manifest moderate to severe acute kidney injury within 12 hours. The urine biomarker test’s investigators believe NephroCheck will give clinicians the early warning signs they need to head off impending cases of AKI, though it remains to be seen whether that hoped-for prevention will bear out in clinical outcomes studies. One important laboratory hurdle to widespread use of the test is that it is performed on a countertop instrument separate from the automated line used for all other urinalyses.

Dr. Uettwiller-Geiger

NephroCheck detects two novel AKI risk biomarkers called urine insulin-like growth factor-binding protein 7 (IGFBP7) and tissue inhibitor of metalloproteinases 2 (TIMP-2).

“TIMP-2 and IGFBP7 provide an early alarm signal, just like a fire alarm, that something may be wrong and the patient requires additional attention,” says Denise Uettwiller-Geiger, PhD, DLM(ASCP), director of laboratory services and clinical trials at John T. Mather Memorial Hospital in Port Jefferson, NY, and a principal investigator in the FDA registration trial for NephroCheck.

Nephrologist and intensivist Kianoush Kashani, MD, principal investigator for the biomarker discovery study and an attending consultant at Mayo Clinic in Rochester, Minn., says the availability of the biomarkers “won’t solve all of our problems but it’s a significant improvement over the status quo. We have been managing these patients blindly.” Changes in urine output or serum creatinine could be subtle or masked by other interventions like fluid resuscitation or use of diuretics, he notes, making it difficult to diagnose AKI at the start.

So exactly how do the two new biomarkers presage acute kidney injury? The renal tubular epithelial cells release IGFBP7 and TIMP-2 when exposed to any number of stressors, including bacterial and drug toxins, John A. Kellum, MD, principal investigator for the AKI biomarker validation trials, said in a talk at last year’s AACC annual meeting. This results in cell-cycle arrest at G1. The cell normally progresses from G1 to S-phase to G2 into cell division and mitosis, he explained. However, he added, “The cell doesn’t want to risk reproduction if its DNA might be damaged or if it may not be in a bioenergetically favorable environment. So it checks itself at G1 before it advances into the cell cycle if it senses anything out there that might be dangerous or injurious.”

“This is very interesting because it gets to the meat of the matter. It’s before injury actually occurs,” said Dr. Kellum, who is a professor of critical care medicine, medicine, bioengineering, and clinical and translational science; vice chair for research in the Department of Critical Care Medicine; director of the Center for Critical Care Nephrology; and associate director for acute illness, Institute for Personalized Medicine—all at the University of Pittsburgh.

If the biomarkers seem to have come out of the blue, they did in a way. That’s because of how the markers were found, which Dr. Kellum says is described in the article reporting the discovery and validation trials (Kashani K, et

al. Crit Care. 2013; 17:R25). Summarizing that information in a recent CAP TODAY interview, Dr. Kellum notes that biomarkers are often discovered through use of model systems. “The simplest type of model system that you could imagine, for example, would be in cancer. So you have a tumor and grind the tumor up and run it through a variety of mass spec and various other types of unbiased discovery to find out whether there would be proteins or other molecules in the tumor that could be used potentially as biomarkers.”

AKI patients don’t often have their kidneys biopsied, which rules out using tissue for studies. “And there’s no animal model that accurately recapitulates clinical acute kidney injury in humans because AKI in humans tends to have a multifactorial cause,” he says.

Because of this heterogeneity, Dr. Kellum and colleagues decided to collect samples from large numbers of people who had very different types of AKI—different exposures, different susceptibilities—to see if they found anything robust in the urine for all of the different AKI etiologies. “We measured about 340 different molecules in the blood and urine, and these two markers were the ones that rose to the top.”

None of the candidate biomarkers except for somewhat known ones like NGAL, KIM-1, and L-type fatty acid-binding protein had ever been associated with kidney injury, Dr. Kellum says. All had been reported on in the cancer or trauma literature, however, as having something to do with the biology of cell stress or damage, he adds. “That’s kind of how we generated the list.”

Following the discovery study, Dr. Uettwiller-Geiger said in the same 2014 AACC session, the assay was created and validated in the Sapphire clinical trial study of 728 patients in 35 sites worldwide. Said Dr. Kellum, “The two biomarkers outperformed all of the other biomarkers that exist out there in the literature.”

The two markers were also found to augment each other. As Dr. Kashani, et al., wrote in the 2013 *Critical Care* article: “…IGFBP7 is superior to TIMP-2 in surgical patients while TIMP-2 is best in sepsis-induced AKI. These differences may underline subtle but important different mechanistic differences between various etiologies of AKI, and the two biomarkers are involved in slightly different pathways.”

The Sapphire study, Dr. Kellum tells CAP TODAY, confirmed that the biomarkers weren’t elevated in patients without AKI who had other acute or chronic comorbidities. The biomarkers don’t “go up in chronic kidney disease unless there is AKI superimposed.”

Other biomarkers included in the study, such as KIM-1 and NGAL, are elevated in AKI. They are, however, also increased in patients with comorbidities, though not as much if the patients don’t also have AKI. Even so, “The noise ratio becomes problematic,” he says.

As for why those two AKI biomarkers are nonspecific, Dr. Kellum explains: “NGAL [neutrophil gelatinase-associated lipocalin], as the name tells you, is a neutrophil-associated lipocalin. So [neutrophils] produce it as well as the epithelial cells in the kidney. Thus, conditions that damage or activate neutrophils can cause it to increase.
“KIM-1 may be a problem because it’s just so sensitive. If one tubular cell gets damaged, maybe that’s meaningless from a clinical perspective in terms of predicting AKI. And yet maybe that’s what we are detecting with a KIM-1 signal. Some markers can be too sensitive and they lose their specificity.”

The researchers further validated NephroCheck in a second study called Opal. Then came an FDA registration trial, the Topaz study, a multicenter U.S.-only clinical study in which a three-member panel clinically adjudicated every case of AKI, Dr. Kellum said. The outcome of that trial showed that “results are even better with a validated, adjudicated endpoint.”

The University of Pittsburgh Medical Center, Mather Hospital, and Mayo Clinic in Rochester are bringing NephroCheck in-house for clinical use. Dr. Kellum expects the test to be available soon at UPMC. Dr. Uettwiller-Geiger and colleagues at John Mather are first introducing the AKI markers educationally through a multidisciplinary committee of key stakeholders, including clinicians and imaging and laboratory personnel. The initial focus is on working with the heart team. Mayo Clinic will implement the test once tests standardizing it for

clinical use at Mayo are completed, Dr. Kashani says.

Paul McPherson, PhD, chief scientific officer for Astute Medical in San Diego, says NephroCheck performs a fluorescence immunoassay that measures both biomarkers in nanograms per milliliter. The Astute140 meter then calculates a numerical AKIRisk Score by multiplying the two markers' concentrations and dividing the value by 1,000 to scale it. Hence the units are reported as (ng/mL)2/1,000, he says. The reportable range for the risk score is 0.04 to 10.

In Europe, Dr. Uettwiller-Geiger says, NephroCheck has two cutoffs. One is 0.3 and the other is 2.0, which has higher specificity. "However, in the United States the assay was FDA cleared with a single 0.3 cutoff," she says.

The testing meter is a "countertop instrument with a small footprint," but NephroCheck has been validated in the U.S. for use only in the central laboratory, she says. Yet since the test produces results in less than 25 minutes, she said in her AACC talk, "it can be done on demand 24/7."

Dr. McPherson

Dr. McPherson says the cartridge, which constitutes one test, is listed for $85 but regular users of "fair amounts" would probably pay about $15 less. The meter costs $4,999, with rental options also available. The test doesn't have a CPT code; its cost would be included in the inpatient diagnosis-related group, Dr. McPherson says, noting that BNP for heart failure was also launched without a CPT code.

Ortho-Clinical Diagnostics and BioMérieux have licenses to develop and market the test for their Vitros and Vidas platforms.

The intended-use population, Dr. McPherson says, is any ICU patient who has or had cardiovascular or respiratory compromise within the past 24 hours. "So that covers many of the patients who go into the ICU." The test can also be performed on such patients in the emergency department. "Let's say the person comes to the ED in septic shock. Even though that patient hasn't been transferred to the ICU yet, they meet all the requirements as they are going to go to the ICU."

The other time it would make sense to test, Dr. Kellum says, is when a critically ill patient who was stable and improving suffers a setback. Perhaps the patient goes into shock or develops a new infection and becomes septic or requires mechanical ventilation.

Dr. Kellum says the NephroCheck test results could be operationalized in the following way for, say, a cardiac surgery patient transferred to the ICU postoperatively and immediately tested:

- A risk score under 0.3. The person would have a "very, very low risk of AKI," he says. In that case, "I can provide the standard of care for that patient without any further consideration because unless something new happens, the risk is really quite low."
- A risk score greater than 0.3 but less than 2. The risk of AKI goes up

about sevenfold in this group. It's absolute risk that's in the range of 12 to 17 to 20 percent, Dr. Kellum says. "It depends a little on what the baseline risk is, so it might be as high as 25 percent but it's certainly not exceedingly high if you're in that middle zone." Yet a clinician is not going to do the same things. "For example, you are not going to give this patient anything that's potentially nephrotoxic. We wouldn't give these patients nonsteroidals. We'd be very careful about fluids and diuretics in that group, much more so than we would with patients with no substantial risk of AKI."

- A risk score exceeding 2. Now the patient has about a one in two risk of developing AKI. "In that population, we really want to sort out what the risk is coming from," Dr. Kellum says. "Maybe the heart function isn't as good as we thought. Maybe we need to get another echo. Maybe there is something happening that we're not sure about—let's investigate further."

Dr. Kellum stresses that NephroCheck wasn't developed to confirm that a patient has AKI. "And no test provides near 100 percent certainty that something will happen."

Determining whether the result is a false-positive "is a tricky thing," he says. "Sometimes the cells will defend themselves successfully so you get an elevation of the biomarkers" but the person doesn't develop AKI.

"Cardiac biomarkers are markers of damage, so you can actually tell a cardiac patient that you just had a little MI because your enzymes are up," Dr. Kellum says. "With NephroCheck, all you can tell the patient is that your kidney was worried, but either because of what we did or what your kidney did, it managed to get through that without any injury."

NephroCheck is not FDA cleared for performing serial measurements to determine whether a patient has increasing or declining biomarker levels. "We can do serial measurements but off-label," says Dr. Kashani, who says the test will be used at Mayo in accordance with the FDA label.

Researchers in the Sapphire validation investigation did collect serial samples for measurement. "So we have a baseline when patients have not actually reached the definitions of moderate to severe acute kidney injury," Dr. Kashani says. "Then we have serial measurement at 12 hours, 24 hours, and then daily for up to seven days or until discharge from the hospital," if sooner than seven days.

Dr. Kashani observes that patients are of several phenotypes. Some recover from AKI rather quickly. "And we expect these markers to identify those patients by decreasing biomarker levels." Some patients go on to have more advanced kidney injury requiring dialysis, and those patients could have higher levels of NephroCheck. The data analysis of NephroCheck trends hasn't been completed, Dr. Kashani says.

Dr. Kellum says NephroCheck is not a test for chronic kidney disease. "But what appears to be true is that if your biomarker levels are really high, not only are you at risk for short-term adverse outcomes but also long-term outcomes."

Anesthesiologist Alexander Zarbock, MD, of the University of Münster in Germany, and colleagues performed a study showing that NephroCheck predicted both AKI and renal recovery in cardiac surgery patients

postoperatively. They selected high-risk study participants by using the Cleveland Clinic Foundation score which correlates with dialysis-dependent AKI after cardiac surgery. This approach makes it possible to increase NephroCheck's specificity, Dr. Zarbock says.

An abstract of the study article says the following: "26 patients (52%) developed AKI. Diagnosis based on serum creatinine and/or oliguria did not occur until 1–3 days after CPB [cardiopulmonary bypass]. In contrast, urine concentration of [TIMP-2]*[IGFBP7] rose from a mean of 0.49 (SE 0.24) at baseline to 1.51 (SE 0.57) 4 h after CPB in patients who developed AKI" (Meersch M, et al. *PLoS One.* 2014;9[3]:e93460).

Dr. Fitzgerald

Robert L. Fitzgerald, PhD, a professor of pathology at the University of California, San Diego, who participated in the Topaz study, believes more clinical studies will be essential before NephroCheck is widely adopted. His laboratory is not planning to use NephroCheck at this time. Having to do the test on site is "a challenge for most labs like ours," he says, "where we have a single big platform. Ninety-five percent of your tests get done on a large automated line, and if the test isn't on the platform, it's going to be a struggle to bring it in-house."

"One concern is that we are becoming overloaded with new biomarkers and everyone promises to do great things," Dr. Fitzgerald says. "In the case of kidney disease, there are several biomarkers that have been touted as being the next great thing and none are in widespread use today." What he views as exciting about NephroCheck is its rigorous validation. And the markers' specificity for AKI differentiates it from the other markers, he says: "There is nothing else that does what they appear to do."

At the University of Münster, Dr. Zarbock and colleagues have a clinical trial underway using NephroCheck to identify patients at high risk for AKI after cardiac surgery. "Biomarker-positive patients are randomized to get either a standard of care or a specific treatment bundle to prevent cardiac-surgery–associated AKI," he says.

Dr. Kellum suspects that early intervention studies will home in on removing nephrotoxic medications. "If I have a patient on a medication that could potentially hurt the kidneys but it only affects a small number of patients in an adverse way, I could either wait for the kidneys to appear to be damaged, which is maybe too late to really stop the drug effectively, or I can test you and if your kidney is sensing stress, stop the drug before the drug actually damages the kidney."

In the meantime, the search for AKI treatments marches on. "Unfortunately," says Dr. Kashani, "most of the completed studies have failed to show significant benefit. We are hoping that NephroCheck can identify patients earlier when they have a higher chance to respond to the interventions."

He notes that several ongoing studies are evaluating different medications or interventions, including anti-inflammatory medications or techniques to remove inflammatory mediators. "A large number of investigators are working on metabolomics and proteomics of urine and plasma during AKI to be able to identify other markers," Dr. Kashani says. "Apart from early diagnosis of AKI, the scientific community tries to come up with markers that can identify the nature, intensity, and location of the injury. I'm hoping that in the future we will be able to individualize treatment for each patient," Dr. Kashani says.

He calls NephroCheck one step forward but not the last step. "Hopefully this is the beginning of progress in the field," with much more to understand and discover, he says. "I am very excited about it."

Dr. Kellum thinks that what researchers are not likely to discover is earlier AKI risk biomarkers. That's because TIMP-2 and IGFBP7 are part of an early defense system used by the cells, he says. "If the cell can't detect it, it's hard to imagine there's going to be any biologic signal. So this is probably as early as it gets, and it's certainly earlier than usable biomarkers in other areas," such as cardiac markers.

"Having markers of stress, which is what these markers are in the kidney, are before injury."n
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